Environmental influence in cyathostominae ecology

M.C.M. Couto[1], S. Quinelato[1], C.N. Santos[1], L.S. Souza[1], I.B.M. Sampaio[2], M.L.A. Rodrigues[1]

[1]Institute of Veterinary Science, Department of Parasitology, Rural Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
[2]Federal University of Minas Gerais, School of Veterinary Medicine, Department of Animal Science, Belo Horizonte, Minas Gerais, Brazil

ABSTRACT: Studies of the survival, recovery and migration of cyathostomin infective larvae in a Bermuda grass (*Cynodon dactylon*) pasture were carried out in the Baixada Fluminense county, Rio de Janeiro state. Fresh feces (± 1 kg) from naturally infected horses were deposited monthly on Bermuda grass. Samples of feces and surrounding grass were collected every seven days, from March 2005 to March 2007, and larva were counted. In the feces, cyathostomin L_3 survived for up to 15 weeks, with higher recovery rates during the rainy period (46 228/kg dried herbage – dh), and on the grass for up to 12 weeks. The recovery of L_3 was greater during the dry period in the grass base (1 868/kg dh) compared to the apex (809/kg dh). The migration of $L_3$from feces to grass varied during the period. Climatic factors, such as temperature and rain, influenced the development and migratory behavior of cyathostomin L_3. With regard to the grass base, significant differences were observed at the different collection times. The results demonstrate that under local conditions animals are at permanent risk since the infective larvae are always present on pasture.

Keywords: cyathostomin; Bermuda grass; infective larvae; survival; development

Strongylide nematodes are commonly found in equines and are parasites of veterinary importance. The Cyathostominae subfamily is highly prevalent in equines in different parts of the world (Ogbourne, 1972; Collobert-Laugier et al., 2002; Anjos and Rodrigues, 2003, 2006).

Temperature and moisture are very important for egg and larvae development in feces and for the migration of L_3 to pastures (Mfitilodze and Hutchinson, 1987; Stromberg, 1997; Baudena et al., 2000; Langrova et al., 2003; Ramsey et al., 2004). Studies of the development, survival and migration of cyathostomin L_3 have been conducted in different parts of the world (Courtney, 1999; Baudena et al., 2000; Langrova et al., 2003; Ramsey et al., 2004; Bezerra et al., 2007). The knowledge obtained about the behavior of the larvae in feces and grass can lead to more effective control strategies for these nematodes. Cyathostominae show resistance to different anthelmintics, and the encysted larvae in the mucous membrane of the host and free-living stages in the pasture constitute a refugia. In Brazil, preliminary studies (Bezerra et al., 2007) have evaluated the influence of temperature and moisture on the free-living stages. The type and characteristics of the grass may influence the migratory dynamics of L_3 (Viana, 1999).

This study investigated the development and survival of cyathostomin larvae over a period of 24 months in feces deposited on a Bermuda grass field, as well as the effects of the time of collection during the day and the climatic conditions on the migration of these larvae.

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MATERIAL AND METHODS

Localization

The experimental plots were located in the W. O. Neitz Parasitology Research Station, Helminthology Laboratory of the Department of Animal Parasitology (DAP) of the Institute of Veterinary Science at the Rural Federal University of Rio de Janeiro (UFRRJ), located at 22°41' South latitude and 43°41' West longitude, at an altitude of 33 m. The pasture consisted mainly of Bermuda grass, but other species of grass were also present (*Cyperus* sp., *Brachiaria* sp.).

The experiment

Fecal samples were obtained from naturally infected horses kept at the DAP. Aliquots of ± 1 kg of fresh feces were deposited monthly in the plot, from March 2005 to March 2007. Fecal and grass samples were collected at regular intervals of seven days until no more L_3 were found. The sampling was done at three different times (8 a.m., 1 p.m. and 5 p.m.). The grass samples came from two distinct regions, 0–20cm (base) and 20–40cm (apex). The feces and grass were processed according to (Bezerra et al., 2007).

The climatic data were obtained from the Seropédica Agricultural Weather Station and the temperature of the soil was measured weekly in the experimental plots. All results were recorded weekly on Excel data plans. Due to an accident no deposits occurred in January 2006.

Statistical analysis

Due to the erratic variation in larva counts, non parametric methods were used to analyze the target variable. The Kruskal-Wallis test ($P < 0.05$) (Zar, 1999) was applied to evaluate the number of L_3 recovered from feces, grass base and apex at different collection times, using the BioEstat program (Ayres et al., 2005).

The Mann-Whitney test ($P < 0.05$) (Zar, 1999) was applied to evaluate the mean values of temperature, rainfall, fecal eggs counts (FEC), survival and recovery of L_3 between the dry and rainy periods, using BioEstat (Ayres et al., 2005). Non inferential multivariate analysis of principal components (Judez, 1989) was applied to study the association of all variables and to facilitate an understanding of the dynamics of L_3 migration. To achieve this, larva counts during a given period of time were given in percentages relative to the period total count.

RESULTS

Meterological data

The mean values of rainfall and air temperature are shown in Figure 1. During the dry season (April to September) rainfall was 187.8 mm and the average temperature was 22.1°C, while during the wet period (October to March) rainfall was 1 919.1 mm and the temperature averaged 25.3°C. Higher temperatures were registered in February 2006 and 2007 and rainfall had its maximum value in February 2006 ($P < 0.05$).

Fecal egg counts

During the experimental period the fecal eggs counts (FEC) varied from 850 to 3 600 in the dry period (Figure 2), and with a mean of 1 421 eggs (± 386.4) and 2 223 (± 947.3) for the rainy period ($P < 0.05$).

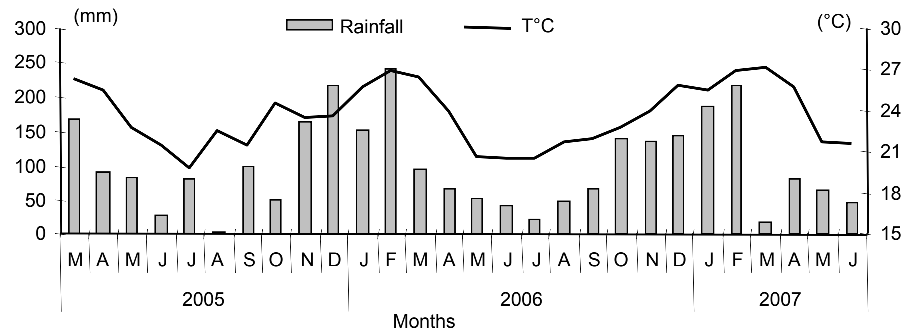

Figure 1. Average temperature and rainfall values from March 2005 to June 2007

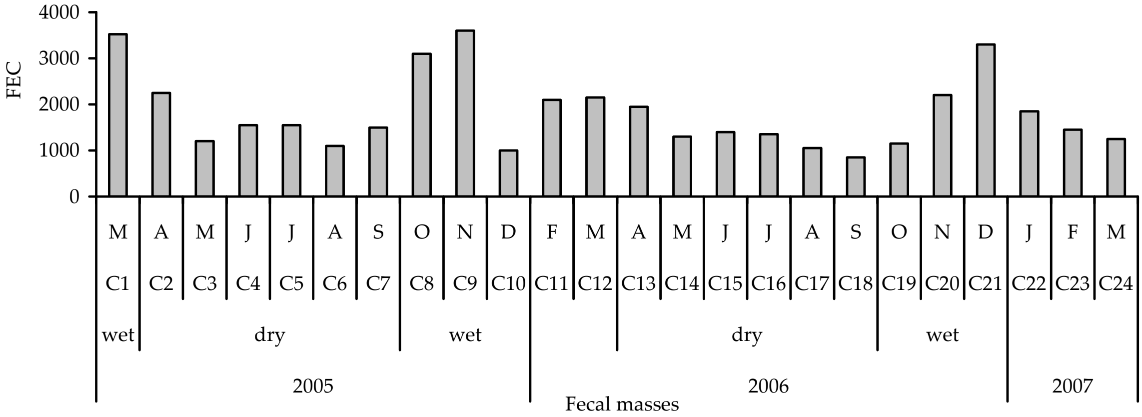

Figure 2. Fecal egg counts (FEC) from March 2005 to March 2007

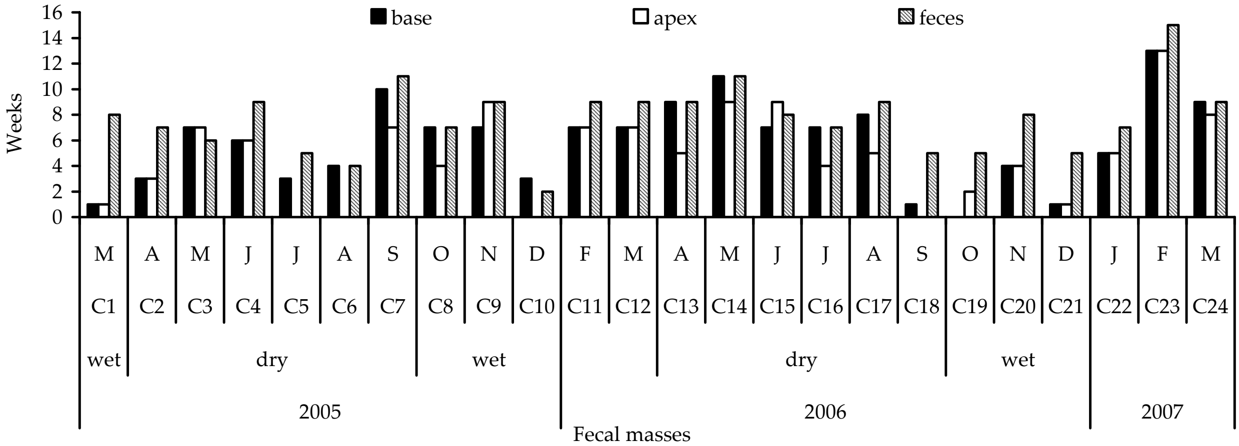

Figure 3. Infective larvae survival (weeks) in feces, grass base and apex

Survival

The survival of infective larvae in feces varied from 2 to 15 weeks, with 15 weeks in the wet period and 11 weeks in the dry period ($P > 0.05$) (Figure 3).

For the grass apex, survival during the rainy period was up to 13 weeks, and in the dry period was up to nine weeks ($P < 0.05$). For the grass base, the maximum survival of infective larvae was 13 weeks during the wet period and 11 weeks during the dry period ($P < 0.05$) (Figure 3).

Recovery

Total. During the rainy period, the mean number of L_3 recovered in feces was 46 228/kg dh, and during the dry period it was 33 723/kg dh, with peaks in May 2006 (C14) and January 2007 (C22) ($P < 0.05$) (Figure 4). In the grass base, peaks were observed in the months of August 2005 (C6) and January 2007 (C22) (Figure 4), with an average recovery in the rainy period of 1 833/kg dh and of 1 868/kg dh in the dry period. For the grass apex, the corresponding recovery during the rainy period was 769/kg dh and during the dry period 809/kg dh, with peaks in June 2005 (C4) and January 2006 (C22) ($P > 0.05$) (Figure 4).

Collection times within a day. Greater cyathostomin L_3 recovery was observed at 1 p.m., 8 a.m. and 5 p.m., for feces, base, and apex of the grass, respectively (Figure 5). Peaks of larvae recovery were noted during rainy and dry periods (Figure 6). There was no significant difference between the collection times ($P > 0.05$) for the feces and grass

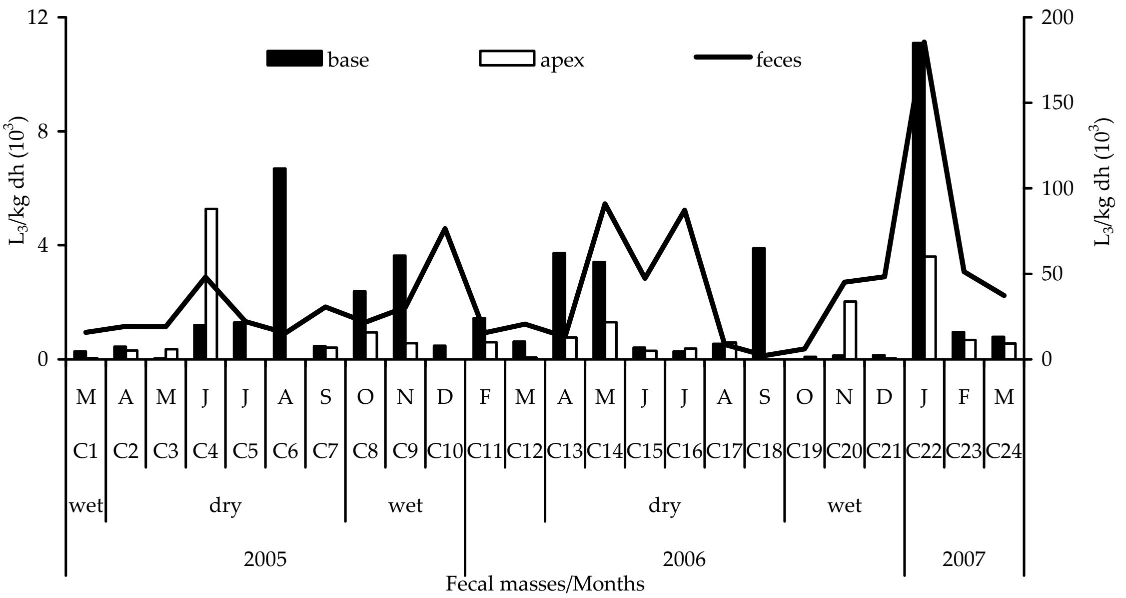

Figure 4. Infective larvae recovery in kg dh (10^3), in feces, grass base and apex

apex. However, a significant difference was found for the grass base ($P < 0.05$) between 8 a m. and 1 p.m. and between 8 a.m. and 5 p.m.

Base vs. apex. The recoveries of L_3 in the base and in the apex of the grass were compared, and the higher value was observed in the base ($P > 0.05$) (Figure 7).

Migration

The migration of L_3 to the grass occurred between weeks 1 and 7. In the rainy period migration to the base took 1–2 weeks and to the apex 1–5 weeks were required. In the dry period, migration varied between 1–7 weeks to the grass base and 1–3 weeks for the apex ($P < 0.05$).

Multivariate analysis of principal components

As expected, the percentage of larvae in feces fell with time after deposition (Figure 8). Rainfall was positively associated with the percentage of larvae on grass. High air temperatures reduced the number of larvae on grass (Figure 8). Similarly, high soil temperature decreased number of larvae in feces.

DISCUSSION

The influence of climatic variables on the migratory behavior and the survival of cyathostomin infective larvae were evaluated over 24 months.

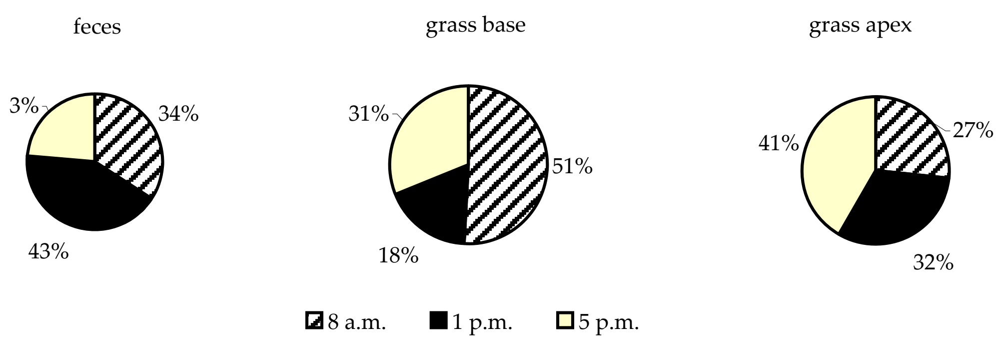

Figure 5. Percentage of recovery of infective larvae at different times of the day in feces, grass base and apex

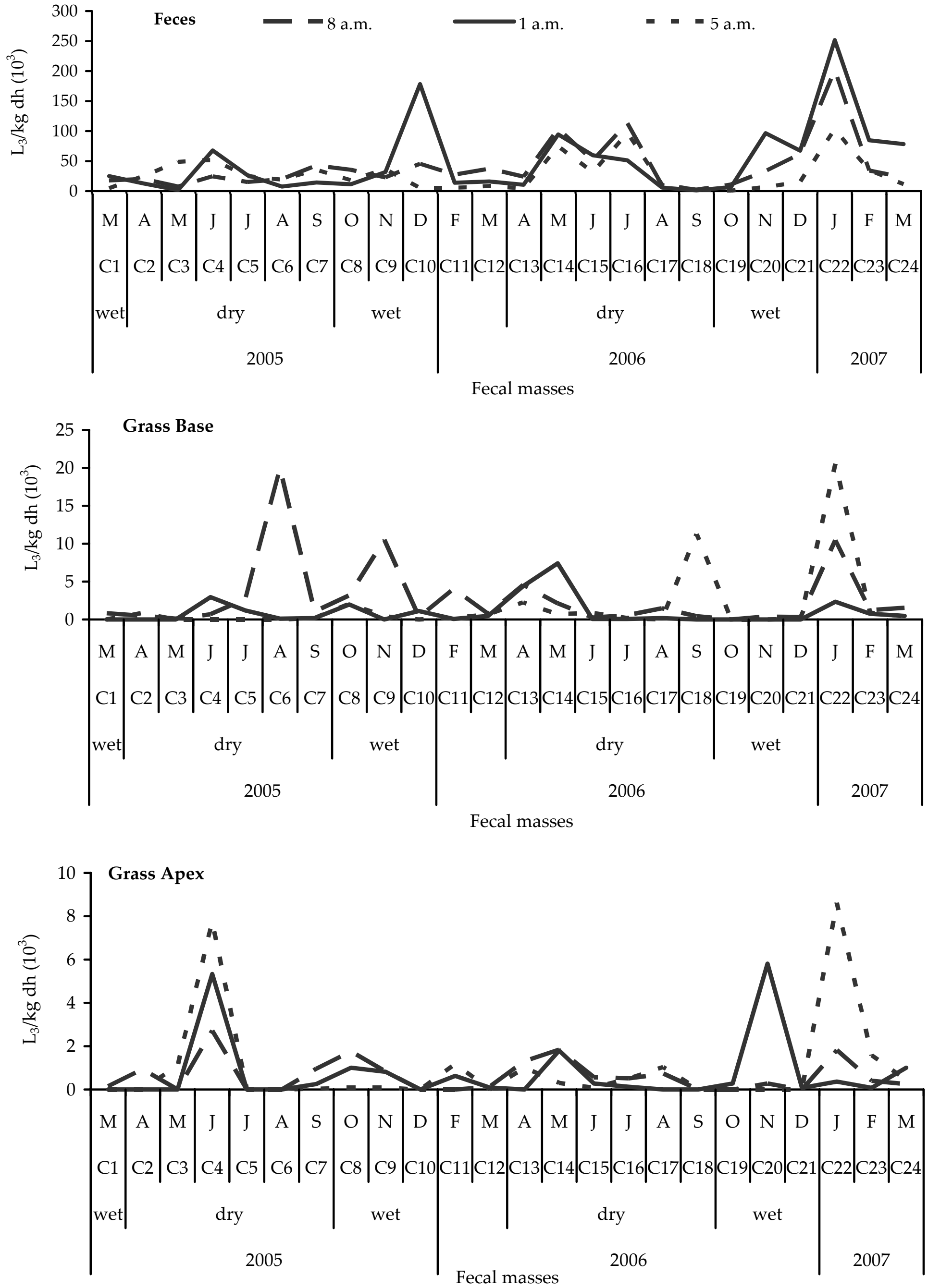

Figure 6. Average of infective larvae recovered in feces, grass base and apex in different times from 2005 to 2007

The climatic conditions of the Baixada Fluminense county in Rio de Janeiro favor the development and survival of cyathostomin free-living stages, because strongyle larvae can develop at temperatures from 8 to 38°C, with soil moistures of more than 30% (Ogbourne, 1972; Mfitilodze and Hutchinson, 1987). In this experiment, even in the hottest months the maximum soil temperature was usually lower than 35°C. Such conditions allowed a high percentage of larvae to survive and develop, in contrast to other studies in Ukraine where a maximum soil temperature of 40°C was observed (Kuzmina et al., 2006).

In certain periods of the year, large numbers of infective larvae tend to remain in the feces, which acts as a reservoir, and do not migrate to the grass. Rainfall and temperature influence the behavior and migration of larvae. This influence has been noted in other larvae ecology studies (Ramsey et al., 2004; Kuzmina et al., 2006).

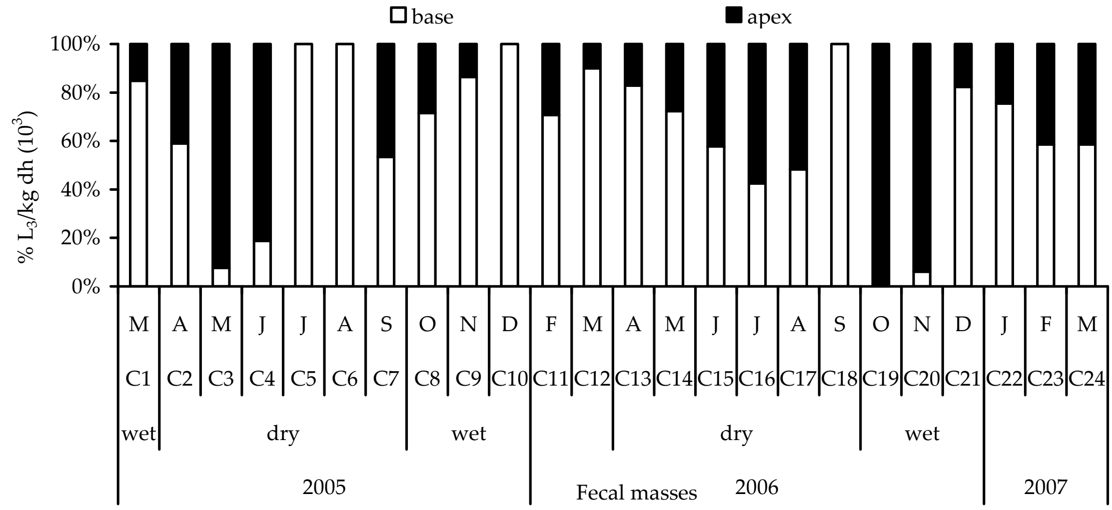

Figure 7. Percentage of recovery of infective larvae in grass base and apex from 2005 to 2007

Heavy rainfall, which occurs frequently during the rainy season (summer), can wash away the L_3 which are close to the fecal mass, spreading the larvae in the pasture, so that the number of larvae recovered in the grass is smaller when compared with the dry period. Therefore, the rainfall is a very important way in which horse cyathostomin larvae are spread on pastures.

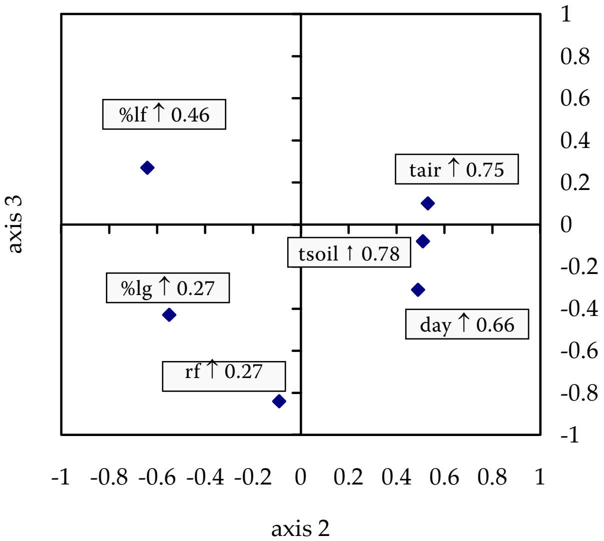

Figure 8. Graphical representation of variables acting in Bermuda grass infective larvae, according to axis 2 and 3. The coordinate and direction of the first axis are represented by the arrow and its value. System inertia = 76%

%lf = percentage distribution of larvae in feces; %lg = percentage distribution of larvae in grass; day = days; rf = rainfall; tair = average of air temperature; tsoil – average of soil temperature

Regarding the influence of temperature and moisture, the largest recovery of L_3 on grass was observed during the mild daytime temperatures (8 a. m. and 5 p.m.). This suggests that at these times animals in pastures are at a higher risk of infection. This finding was not observed in preliminary studies conducted in the same area (Bezerra et al., 2007); however, it agrees with observations cited in a study conducted in the Czech Republic, which pointed out the importance of dew for the migration of L_3 (Langrova et al., 2003).

Animals kept on pastures in the Baixada Fluminense region may always become infected, therefore, as L_3 are present on the pasture throughout the year.

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Corresponding Author:

Maria de Lurdes de Azevedo Rodrigues, Rural Federal University of Rio de Janeiro, Km 7 BR 465, Seropédica, RJ-CEP 23890-000 Brazil

Tel. + 55 21 2682 1617, e-mail: lurdesar@ufrrj.br